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Does the evolution of increased competitive ability hypothesis explain invasion? Comparing native and non-native populations of *Plantago lanceolata*

Izzadora Dean

Project Advisor: [Mick Hanley](#), School of Biomedical & Biological Sciences, University of Plymouth, Drake Circus, Plymouth, PL4 8AA

Abstract

1. The evolution of increased competitive ability (EICA) hypothesis proposes that release from enemy regulation in new ranges results in changes in selective pressure, producing poorly defended, rapidly growing phenotypes. *Plantago lanceolata* is not exposed to intense mollusc herbivory in its non-native North American range, therefore post-invasive evolutionary change may be observed in these populations.
2. Seeds from eight populations, four invasive and four native, were collected and grown in glasshouses. Seedling acceptability to the mollusc *Helix aspersa* was measured, along with recovery after artificial cotyledon removal, seed mass and seedling mass at 14d old.
3. No difference in acceptability was found, and recovery from cotyledon damage missed statistical significance. The results do not support the EICA hypothesis, with variation between populations likely to be due to factors such as differing selective pressures or founder effects.
4. No link between acceptability and recovery was evident, suggesting no trade-off occurs between these two traits.
5. Seed and seedling mass was smaller in invasive provenance populations ($P < 0.01$, $P = 0.053$). This may indicate the different environmental pressures in each provenance, resulting in different life history strategies. A correlation between the two exists ($P < 0.05$). In seedling and seed mass, the main contribution to the smaller invasive provenance populations came from the reduced sizes of the Carson population. However, no links between seed mass and acceptability or recovery were found.
6. *Synthesis*. To develop an understanding of the mechanisms underlying botanical invasions, the assimilation of hypotheses must occur, to gain insight as to the relative importance of disturbance, competition, plant traits and enemy release.

Key-words: EICA, enemy release, *Helix aspersa*, herbivory, invasive species, palatability, plant defence, post-invasion evolution, seed mass, trade-offs

Introduction

Biological invasions have been widely observed in all ecosystems and are considered second only to habitat destruction in the risk they pose to biodiversity (Vitousek *et al.* 1997). However, only a small proportion of non-indigenous species are able to establish, and fewer still become a significant threat to native ecosystems (Mack *et al.* 2000). Understanding what makes a species invasive is essential to devising methods of control, though further research is required (Kolar and Lodge 2001).

Many studies have observed a tendency for invasive plant species to grow larger than individuals found within its original environment (Blossey and Nötzold 1995; Leger and Rice 2003; Erfmeier and Helge 2004). Several hypotheses have been proposed to explain this (Blumenthal 2006). The importance of enemy regulation in moderating plant populations has been highlighted (Keane and Crawley 2002). The seedling stage is the most important in ascertaining an individual's establishment and reproductive potential, with herbivory often being the greatest cause of seedling mortality (Moles and Westoby 2004). New environments can lack associated predators and pathogens, perhaps allowing for swift population build up and spread (Herms and Mattson 1992; Müller-Schärer, Schaffner and Steinger 2004). This has been demonstrated in many experiments involving non-indigenous invasive species (Mitchell and Power 2003; DeWalt, Denslow and Ickes 2004; Agrawal *et al.* 2005; Adams *et al.* 2009).

Plants are considered to make a trade-off between two contrasting strategies in response to herbivory – to grow quickly to add the photosynthetic area, or to invest in protecting their edible photosynthetic parts (Herms and Mattson 1992). A hypothesis developed with this in mind is that “predator release” exerts a selective advantage on phenotypes with greater investment into competing effectively for resources, such as by vigorous growth, rather than costly resistance traits that confer protection from the absent herbivores (Blossey and Nötzold 1995; Müller-Schärer, Schaffner and Steinger 2004). This shift in allocation from resistance to vigour is the centrepiece of The Evolution of Improved Competitive Ability (EICA) hypothesis (Blossey & Nötzold 1995).

The assumptions of the EICA hypothesis are that species naturalised to new ranges will grow larger than the native range ecotypes, and that specialised herbivores (often absent from the new range) will perform better on the invasive provenance (IP) populations than the native provenance (NP) populations (Blossey & Nötzold 1995). The reason specialist herbivores are emphasised is that producing chemicals to deter a specialist has a greater fitness cost than those that resist generalist herbivory (Joshi and Vrieling 2005). Studies conducted to test the EICA hypothesis have failed to produce consistent results, with support being found in some cases (Blossey and Nötzold 1995; Leger and Rice 2003; Siemann and Rogers 2003), but limited or mixed in others (Willis, Memmott and Forrester 2000; Maron and Vilà 2001; Vilà *et al.* 2003; van Kluenen and Schmid 2003; Agrawal and Kotanen 2003; Maron, Vilà and Arnason 2004; Buschmann, Edwards and Dietz 2005; Joshi and Vrieling 2005).

In Eurasian grasslands, *Plantago lanceolata* is a very common grassland herb (Rose 2006), and has established weedy populations worldwide through human activity

(Barton 2007). In Europe, molluscs are an important predator in grasslands and *P. lanceolata*'s success in grazed communities is due in part to its defences (Hulme 1996). Terrestrial molluscs are considered more abundant in the milder, wetter climate of Europe (Blumenthal 2006), thus according to the EICA hypothesis defence against them would not be heavily selected in the drier North American climate (Blossey and Nötzold 1995).

Helix aspersa is a species native to the British Isles and much of Europe, and though it has been accidentally introduced to the North American continent it does not sustain a population size similar to much of Western Europe due to climate differences and pest elimination and quarantine efforts (Capinera 2001). The EICA hypothesis predicts that, given the absence of mollusc predation in mediating USA populations of *P. lanceolata*, these ecotypes would possess fewer deterrents and show greater vulnerability to attack than their European conspecifics (Blossey and Nötzold 1995). Additionally, the IP plants should attain greater size and biomass, due to the reallocation of resources (Blossey & Nötzold 1995).

Seed size is often seen as a good indicator of the life history strategy likely to be employed by a plant (Harper, Lovell and Moore 1970). Therefore seed size may be expected to change as a species enters a new range to reflect a change such as that proposed by the EICA hypothesis (Buckley et al. 2003).

Typically, seed size is often linked to the balance between offspring number and offspring fitness (Harper, Lovell and Moore 1970; Smith and Fretwell 1974). Small seeds may be produced in greater numbers than larger seeds, though small seeds are often limited to recently disturbed communities, most successfully under high nutrient regimes and low stress (Burke and Grime 1996; Baraloto, Forget and Goldberg 2005). In contrast, larger seeds are better at establishing in undisturbed communities, often of a late successional stage (Burke and Grime 1996; Hanley 1998). This is supported by evidence of greater capability to overcome low light levels (Foster and Janson 1985), deep leaf litter (Molofsky and Auspurgner 1992), drought (Leishman and Westoby 1994) and resource limiting soils (Milberg, Pérez-Fernández and Lamont 1998; Walters and Reich 2000), though some works question the true value of the trade-off (Leishman *et al.* 2000; Moles and Westoby 2004). Large seeds are also observed to greater invest in secondary metabolites to reduce appeal to herbivores (Westoby *et al.* 1997; Hanley and Sykes 2010), and higher tolerance (recovery ability) to herbivory (Armstrong and Westoby 1993).

Invasive provenance conspecifics have been reported to produce more seeds than their NP counterparts (Noble 1989). Therefore smaller seeds should be observed in IP environments, to better increase spread, particularly in communities prone to disturbance (Burke and Grime 1996). However, making such an assumption must be done with care, as selective pressures vary between environments, and impacts on the maternal plant may result in the production of larger or smaller seeds accordingly (Buckley et al. 2003).

In this study, feeding preferences of *Helix aspersa*, seedling mass (at 14d), seed mass and seedling recovery from cotyledon damage (a tolerance measure) were observed in four native provenance (NP) and four invasive provenance (IP) ecotypes of *Plantago lanceolata*. There are three questions addressed by the study. (i) Are the

IP populations more susceptible to attack by the mollusc predator *Helix aspersa* than the NP populations? (ii) Do the IP populations grow larger than the NP populations? (iii) Do trade-offs occur between frequency of mollusc attack, tolerance and seedling growth? (iv) Can seed size be used to predict trade-offs between traits important in invasions?

Materials and Methods

Plant characteristics

Plantago lanceolata L. (Plantaginaceae) is a rosette-forming annual or facultative perennial whose native range reaches from east Spain to northern and central Asia, including the British Isles (Rose 2006; Barton 2007). It can be found on the North American continent, where it is considered a weed (Barton 2007). Within North-Western Europe its seedling establishment is at least partially regulated by mollusc species (Hanley, Fenner and Edwards 1996). They produce iridoid glycosides, primarily aucubin and catalpol, which act as deterrents to molluscs (Dirzo 1980). The production of iridoid glycosides is genetically determined, can be induced via herbivory and increases in concentration with age (Bowers and Stamp 1993; Barton 2008). It is a species that is successful in grassland environments subjected to high rates of invertebrate grazing, implying the role of these secondary metabolites in adults in deterring herbivores (Hulme 1996).

Seed collection

Seeds of *Plantago lanceolata* were collected from four locations in Europe and four in North America between the dates of August and October 2008. The European collections came from Devon, England (Taunton, 51°01'N 3°03'W), France (Samouillan, Haute Garonne, 43°15'20"N 0°55'58"E), Germany (Leipzig, 50°20'13"N 12°24'20"E), and Shropshire, England (Quatford, 52°30'32"N 2°23'12"W). North American populations came from Washington (Carson, 45°45'N 121°50'W), Wisconsin (Waterford, 42°46'N 88°11'W), Oregon (Ophir, 42°35'N 124°23'W) and California (Elkmeadow 41°19'N 124°02'W).

Herbivore choice and collection

For the feeding preference tests *Helix aspersa* was selected. This choice was made due to its large wild population and ease of collection and storage. Sixty individuals were collected in the spring/summer of 2009, with the remainder being collected throughout November 2009. These all came from Plymouth, Devon, UK. The snails were kept in containers of various sizes and stored at 15°C with a 12hrs light: 12hrs darkness cycle in a stable temperature room, installed by HBCL Coldstores Ltd with temperature control by Bradley Refrigeration. Snails were fed on a diet of various greens, such as white and Savoy cabbage and allowed at least 7d to acclimate to these conditions. They were subjected to starvation for 3-6d before each acceptability test commenced. Snails were not standardised by mass, as it has been shown that biomass does not relate to the quantity of plant matter consumed (Hanley, Bulling and Fenner 2003).

Seedling acceptability

Plantago lanceolata seeds were set up to germinate on 90 mm Petri dishes with Whatman No. 2 filter paper saturated with water at the bottom. These were placed in the stable temperature room at 15 °C with a 12hr light: 12hrs darkness regime,

described previously. After 4d seeds showing radical emergence no greater than 1cm were selected for planting.

Plastic pots (50 x 50mm) were filled with John Innes No. 2 compost and pairs of *P. lanceolata* seeds originating from the same location planted opposite one another. These were kept in a glasshouse with a mean daily temperature varying between 23.6°C (SE \pm 0.7) maximum and 10.9°C (SE \pm 0.4) minimum. After 7d, two germinated seeds of the *Lactuca sativa* ("Little Gem" Lettuce) were planted in each pot in the opposite corners to the *P. lanceolata* seeds. These were planted 7d later as *P. lanceolata* at 14d and "Little Gem" at 7d represent approximately the same ontogenic stage of development. The 'Little Gem' individuals were used as an index species, against which the relative acceptability of each ecotype of *P. lanceolata* could be compared (Fenner, Hanley and Lawrence 1999).

Acceptability tests were conducted 13-15d after *P. lanceolata* was planted. Five pots representing a single population were placed in trays (350 x 220 x 50 mm) filled with compost, reaching the upper lip of each pot. They were arranged with one in the centre and one occupying each corner. Two *H. aspersa* individuals were placed in each tray and prevented from escape using a plastic propagator lid (350 x 215 x 70 mm). The tests would begin between 4 and 5pm in an evening and finish between 9 and 10am the following morning, as *H. aspersa* has nocturnal feeding habits (ME Hanley. Pers Comm). The quantity of plant material eaten from each plant was assessed and placed into a category indicating a percentage range.

This data was used to produce Acceptability Index (AI) (Fenner, Hanley and Lawrence 1999), which indicates the comparative preference of *P. lanceolata* to the 'Little Gem' lettuce. The index was calculated using the following formula

$$\text{AI per tray} = \frac{\text{Number of } P. \textit{lanceolata} \text{ seedlings attacked}}{\text{Number of test } + P. \textit{lanceolata} \text{ seedlings attacked}}$$

'Attack' was defined in two different manners. The first used all damage inflicted by herbivory, no matter how much. The second classified 'attack' as damage inflicted by herbivory in excess of 25% damage. This second definition was chosen to eliminate data with very minimal data from collection, under the assumption that seedlings suffering small amount of damage were unfavoured by *H. aspersa* individuals. Data analysis was conducted on both figures.

All populations were replicated between six and nine times with the exception of Taunton, of which only four replicates were obtained. As such, Taunton was excluded from any acceptability statistical analysis.

Seed mass

Due to the small mass of individual seeds, they were measured in groups of 10 for 15 replicates. Seeds of each ecotype were taken and mass recorded using a Precisa 180A (Balances Ltd) balance. The seeds were chosen at random, extracted and cleared of additional debris using a fine paintbrush. The seeds previously weighed were kept separate to ensure single seeds were not weighed multiple times.

Seedling mass

Fifteen individuals from each population were grown in January 2010. Seeds of each population were germinated as described previously and planted in pots (50 X 50mm) in the greenhouse with a mean daily temperature of 21.7°C (SE \pm 1.4) maximum and 8.2°C (SE \pm 0.7) minimum. These were arranged in lines from front to back, to ensure each population received comparable amounts of sunlight. They were harvested at 14 days old. This procedure involved carefully decanting the plant and soil material and gently rubbing the soil with fingertips to remove the majority of the soil from the roots without losing biomass. The plants were washed of remaining soil using a mister containing tap water. These were put in envelopes and placed inside an oven (Memmot D 06062 Modell 600) at 60°C for 70 hrs. After the drying period, the samples were weighed using a balance (Mettler AT201). Fifteen replicates were acquired for every ecotype, excluding Taunton, of which there was not enough viable germination.

Tolerance to damage

Plantago lanceolata individuals were allowed to germinate in January and February 2009 on wetted Whatman no. 2 filter paper in Petri dishes, then placed into a dark incubator at 15°C. After radical emergence, 60 individuals of each ecotype were planted in John Innes No. 2 and allowed to grow in a glasshouse with a mean daily temperature varying between 23.8°C (SE \pm 1.3) maximum and 11.4°C (SE \pm 0.6) minimum. At 14d old, 30 individuals of each group received cotyledon removal. Artificial removal was chosen over natural as it is easier to manipulate and produces greater uniformity between damaged individuals, though components of natural damage that may influence plant response are missing, such as herbivore saliva (Tiffin and Inouye 2000). At 35d old, 15 of each treatment were harvested and, washed of compost using a mister and oven-dried at 60°C for 24hrs to attain a measure of dry-weight biomass.

Given the natural variation in plant size, it was necessary to reduce its influence in the data. This was achieved by calculating a mean percent recovery for each population:

$$\text{Tolerance} = D(100 / C)$$

D represented the mean mass of seedlings at 35 d after cotyledon removal at 14 d and C representing mean mass of undamaged control seedlings. Using this index was an attempt to rule out the variation that would occur in seedling mass between and within population, so as to acquire the best representation of recovery possible.

Results

Data analysis

All data were arcsine transformed prior to data analysis (Dytham 2003). A Levene's test and Kolmogorov-Smirnov test was used to assess the normality of the data (Dytham 2003). The difference in acceptability was assessed using a General Linear Model ANOVA, in which data from all populations was clumped into the categories IP (invasive provenance) and NP (native provenance). Though it was desired to assess differences between populations within and between the IP and NP, sample sizes were consistently small and highly nested, thus it could not be achieved. Hypotheses regarding the relationships between two variables were addressed through Pearson correlations (r), using mean data for each population.

Table 1. The mean values of *P. lanceolata* traits between invasive and native populations. Means for invasive and native provenances are indicated in italics. *Information derived from 3 data points.

Population of origin	Seedling Acceptability (\pm SE)	Tolerance (% Recovery)	Mean seed mass (mg) (\pm SE)	Mean 14d seedling mass (mg) (\pm SE)
<i>Native Populations</i>	<i>0.672 \pm 0.07</i>	<i>23.4</i>	<i>15.1 \pm 0.49</i>	<i>1.36 \pm 0.06</i>
Samouillan	0.694 \pm 0.12	21.2	13.34 \pm 0.46	1.45 \pm 0.11
Leipzig	0.81 \pm 0.1	20.3	11.73 \pm 0.52	1.39 \pm 0.09
Quatford	0.518 \pm 0.16	25.1	20.2 \pm 0.62	1.72 \pm 0.08
Taunton	0.556 \pm 0.206 *	26.8	15.13 \pm 0.47	n/a
<i>Invasive Populations</i>	<i>0.643 \pm 0.07</i>	<i>26.8</i>	<i>11.7 \pm 0.4</i>	<i>1.52 \pm 0.07</i>
Carson	0.775 \pm 0.149	19.1	7.55 \pm 0.31	0.66 \pm 0.06
Waterford	0.638 \pm 0.139	36	11.74 \pm 0.21	1.54 \pm 0.08
Elkmeadow	0.713 \pm 0.106	20.8	15.67 \pm 0.4	1.86 \pm 0.13
Ophir	0.438 \pm 0.137	31.2	12.03 \pm 0.35	1.38 \pm 0.1

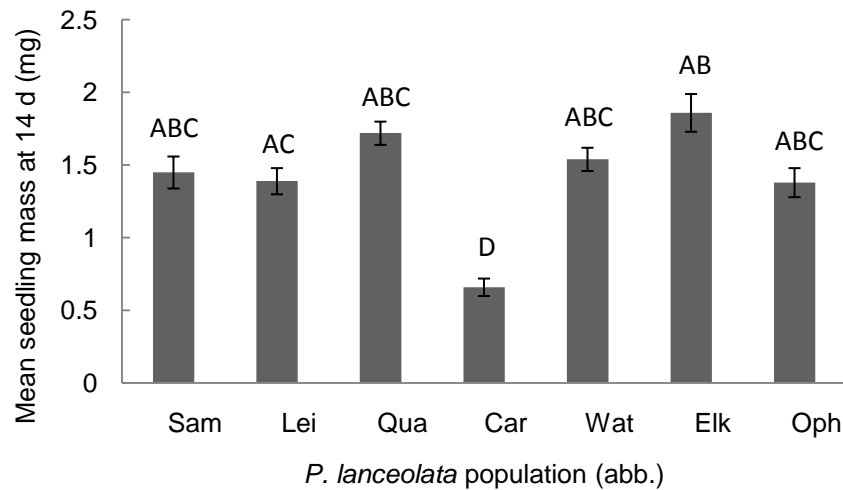


Figure 1. The mean difference (\pm SE) in seedling mass after 14d in *P. lanceolata* from seven populations. When analysed together, difference between continents was close to but not statistically significant ($F_{1,103} = 3.82$, $P = 0.053$). Differences between populations was significant ($F_{6,98} = 10.15$, $P > 0.001$), with Carson being a major contributor to this result.

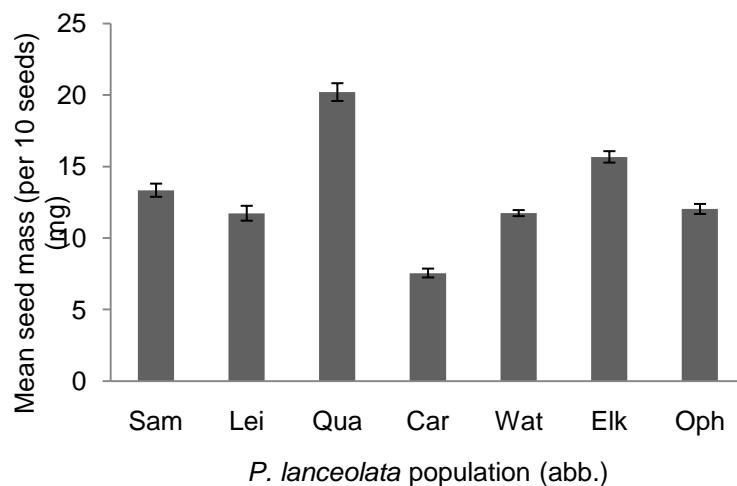


Figure 2. The mean seed mass per ten seeds (\pm SE) across seven populations of *P. lanceolata*. Differences between continents was considered significant ($F_{1,104} = 21.25$, $P < 0.01$), though there was no overall trend of similarity within the two groups ($F_{6,98} = 85.86$, $P < 0.01$)

Table 2. Results of Pearson's correlation of the strength of relationship between traits across all plant populations

	<i>r</i>	DF	P-value
Tolerance x Acceptability Index	0.058	5	0.901
Seed mass x Acceptability Index	0.243	5	0.6
Seed mass x Tolerance	0.109	6	0.797
Seedling mass x Acceptability Index	0.598	5	0.156
Seedling mass x Tolerance	0.293	5	0.524
Seed mass x Seedling mass	0.842	5	<0.05

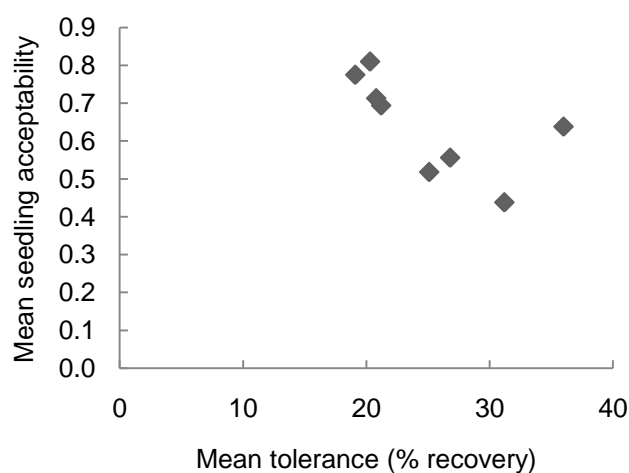


Figure 3. The tolerance (% recover after damage when compared to controls) and acceptability in populations of *P. lanceolata*. There is no statistically significant relationship (Pearson's $r = 0.058$, $DF = 5$, $P = 0.901$). The Waterford population are represented by the deviation from a potential negative trend.

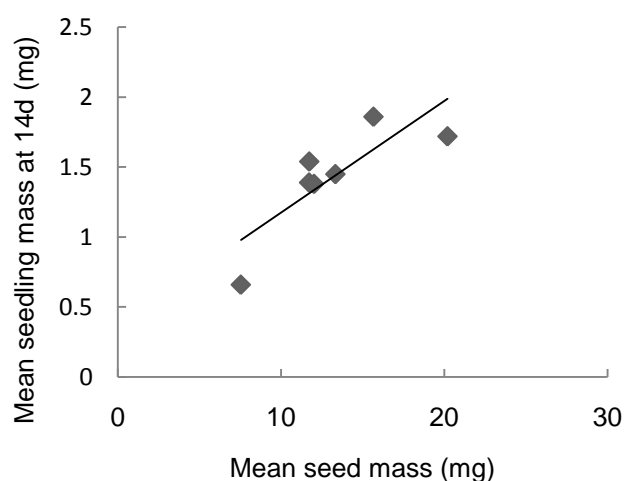


Figure 4. The mean seed mass (per 10 seeds) and mean seedling mass at 14d in populations of *P. lanceolata* (Pearson's $r = 0.842$, $DF = 5$, $P < 0.05$)

Seedling acceptability

No significant difference was found between the acceptability of IP and NP *P. lanceolata*, when analysis was conducted on data from both definitions of 'attack', ($F_{1,55} = 0.65$, $P = 0.423$) ($F_{1,55} = 1.11$, $P = 0.296$) neither was it found between the sample populations ($F_{7,49} = 0.84$, $P = 0.557$) ($F_{7,52} = 0.71$, $P = 0.666$). Only +25% damage definition data listed in the results section (Table 1, Table 2). The lack of difference in palatability and, by proxy, secondary metabolite concentrations between both continents and populations suggests that *P. lanceolata* has not responded to changes in environmental pressures as predicted by the EICA hypothesis (Blossey and Nötzold 1995).

Seedling mass

The second assumption of the EICA hypothesis states that competitive ability will increase in a new range, and this would be evident in plant mass (Blossey and Nötzold 1995). *Plantago lanceolata* IP populations have smaller seedlings than their European counterparts, though this result was not significant and the Carson population contributed greatest to this (Fig. 1, Table 1). The lower mass is opposite to what is predicted by the EICA hypothesis (Blossey and Nötzold 1995). This suggests that genetic differences exist between populations, as is most evident in Carson, but it is not explained by the EICA hypothesis. It is more likely due to other selective pressures in locations regardless of provenance, such as competition (Buschmann, Edwards and Dietz 2005).

Seed mass

Seed mass differed considerably between continents, with invasive populations possessing on average smaller seeds (Table 1). However, between populations there was no overall trend of IP seeds being bigger than NP seeds, with much variation regardless of IP or NP origin (Fig. 2). This may be a reflection of the differences in environmental pressures that the parent plants (from which seeds were collected) were subjected to (Fenner and Thompson 2004).

Analysis of trade-offs

No trade-off between seedling mass and acceptability was found in *P. lanceolata* (Table 2). The EICA hypothesis highlights the importance of changes of investment between these two traits, yet this studies results that such a trade-off does not exist. Tolerance did not show a relationship with seed mass (Armstrong and Westoby 1993) (Table 2). These results suggest that in *P. lanceolata* the benefits of greater seed mass are limited to seedling growth. The observation that seedlings which grow larger tend to invest less into chemical defence (Hanley and Sykes 2010) is also unsupported in *P. lanceolata* (Table 2). It has previously been observed that seed size and life history strategies are not as strongly linked as assumed (Leishman *et al.* 2000).

No relationship was determined between acceptability and tolerance (Fig. 3). Data suggested a negative correlation between the two traits, though the Waterford population seems to detract vastly from this potential trend. Why this is so different is unclear. Previous work has indicated that no trade-off is evident in *P. lanceolata*, though this focused on changes through age (Barton 2007).

Plantago lanceolata seedlings have been noted as phenotypically plastic, growing more vigorously in response to herbivory (Barton 2008). It was considered a possibility that *P. lanceolata* may express a constitutive trait for higher growth at the cost of reduced plasticity in more predictive environments (Schlichting 1986). However, the data does not indicate that such a trade-off occurs ($r = 0.293$, $P = 0.524$). The lack of such a trade-off has been demonstrated previously (van Kleunen and Schmid 2003).

It would be interesting to note whether tolerance to herbivory has changed between NP and IP population regardless of lack of changes in acceptability given the supposedly low cost of resistance traits in some species (Strauss *et al.* 2002), but a lack of replicates made a statistical comparison impossible. However, Table 1 suggests greater similarity between NP populations than between the IP populations,

Seed mass is viewed as a good indicator of the favoured life history strategy of an organism (Hanley 1998). Pearson's correlation indicated a positive link between seed mass and seedling mass (Table 2; Fig. 4), implying the benefits conferred to competitive ability in these populations, aiding establishment under high competition, as often seen in late successional communities (Burke and Grime 1996; Hanley 1998). Much of the seed and seedling mass analysis received much weight from the Carson population (Table 1).

Discussion

Acceptability and seedling mass between provenances

The EICA hypothesis is concerned with changes in resource allocation in a species when it enters a new environment, leaving specialist predators behind in its native range (Blossey and Nötzold 1995). The two basic assumptions are that plants from a new range will acquire a higher biomass than their native conspecifics, and that they will be more palatable to herbivores from their native range (Blossey and Nötzold 1995). The difference in seedling mass was opposite to that predicted by the EICA hypothesis. Plants from the IP populations were no more palatable than the NP individuals, breaking the second assumption (Blossey and Nötzold 1995).

This study was conducted under the impression that *P. lanceolata* had escaped herbivory in North America. However, in North America there are a number of generalists recorded to feed upon it, and the specialist *Junonia coenia* uses its iridoid glycosides as oviposition stimulants (Bowers and Stamp 1993). Thus the uniformity between the *P. lanceolata* populations may be due to a lack of selective pressure to reallocate resources to increased competitive ability. Previous experiments have highlighted that release from a specialist is most likely to produce IP and NP difference which correspond with the EICA hypothesis predictions (Bosssdorf *et al.* 2004), and molluscs are regarded as being generalist herbivores (Wolfe 2002). As such, acceptability for *H. aspersa* may not represent changes in specialist defensive metabolites. Other regulators will exist for *P. lanceolata* such as fungi and soil pathogens, which are often highly specialist and rarely switch hosts (Agrawal *et al.* 2004). Whether these are present in the new range and what contribution they have had in establishment is unclear. A greater understanding of the impacts of these regulators is needed, as well as evaluation of the contribution of specialists and

generalists to enemy release (Maron and Vilá 2001; Buschmann, Edwards and Dietz 2005).

Plantago lanceolata fails to provide evidence for the EICA model. The importance of *P. lanceolata* as an invasive is considered low (Cal-IPC 2006), and there is doubt that significant evolutionary change can occur in less than two centuries in a new range (Keane and Crawley 2002). Given the short duration spent on the North American continent, *P. lanceolata* may not have had the opportunity to express trade-offs, particularly if selection pressure is weak due to the potentially incomplete release from enemy regulation (Bowers and Stamp 1993). Founder effects may have limited the genetic variability of the IP populations, thus contributing to the differences between these populations (Amsellem *et al.* 2000; Sakai *et al.* 2001; Colautti *et al.* 2004).

Trade-offs in invasive success

The EICA assumes a trade-off occurs between seedling mass and acceptability, expressed as a positive relationship, though this was not found in *P. lanceolata*. As acceptability was used as a surrogate for concentration of secondary metabolites, these results do not support the suggestion that IP and NP ecotypes would show a difference in growth and resistance trade-offs (Blossey and Nötzold 1995). Other works have also failed demonstrated a link between the two (Maron, Vilá and Arnason 2004; Buschmann, Edwards and Dietz 2005). Although acceptability did not vary between provenance or populations, seedling mass did. Seedling mass was linked to seed mass, but seed mass did not express a relationship to acceptability, implying that differences in seed and seedling mass are responding to other environmental pressures, or could be linked to founder events or unmeasured selection pressures (Amsellem *et al.* 2000; Sakai *et al.* 2001; Colautti *et al.* 2004).

The lack of evidence for a trade-off between seedling acceptability and tolerance may contradict popular theories on how plants allocate limited resources (Herms and Mattson 1992; Strauss *et al.* 2002). The variability between the tolerance of IP plants is higher than NP plants, suggesting changes in response may have occurred (Table 1), but the nature of them can only be speculated.

It has been suggested that the EICA is only important to species that relied heavily on resistance traits, rather than compensation strategies (Blumenthal 2006). Given *P. lanceolata*'s plastic compensation strategy (Barton 2008), this may explain the lack of support it provides.

Seed mass and invasive traits

Seed size is can be used as an indicator of the life history strategies likely to be employed by the plant (Harper, Lovell and Moore 1970). The large variation in seed mass between provenances and populations would signal these differences, but the only variable in the study that any relationship could be found with seed mass was seedling mass, with small seeds producing small seedlings. There were no changes in acceptability or tolerance in relation to seed size, indicating that any changes in life history strategy occurring had little or no relation to seed size.

The smaller seeds in IP populations possibly reflected a strategy not examined in this study. Small seeds are known to be produced in greater numbers than large

seeds (Burke and Grime 1996), and invasive plants have been reported to produce more seeds than their NP conspecifics (Noble 1989). Therefore the lower seed mass may be linked to the strategy of increasing reproductive to hedge bets that a seed can take advantage of low competition and high resource availability, a common scenario in high disturbance communities (Burke and Grime 1996; Smith and Fretwell 1974; Moles and Westoby 2004). North America has been suggested to observe high disturbance since European colonisation (Mack 1989). However, seed sizes (and, subsequently, seedling biomass) may be influenced by conditions in the place of collection affecting parent plants and, consequently, seed size (Fenner and Thompson 2004). Other qualities indicating adaption for early successional communities include high relative growth rate (RGR) (Bloor and Grubb 2003), though this was not measured in this experiment.

Why such a dramatic difference in the Carson population exists can only be speculated. It may be related to founder events (Amellem *et al.* 2000; Colautti *et al.* 2004) or maternal effects (Fenner and Thompson 2004).

The lack of evidence for a link between tolerance and acceptability indicates that assumptions on some plant characteristics cannot be made lightly. The strength of the seed size/life history strategy assumption has been previously brought under scrutiny, with many taxonomic groups defying generalisations, a warning against applying seed sizes alone to make assumptions in invasive studies (Leishman *et al.* 2000; Buckley *et al.* 2003; Moles and Westoby 2004).

What is the model missing?

Many other studies have tested this hypothesis and produced results that vary widely (Willis, Memmott and Forrester 2000; Maron and Vilà 2001; Leger and Rice 2003; Siemann and Rogers 2003; Vilà *et al.* 2003; van Kluenen and Schmid 2003; Buschmann, Edwards and Dietz 2005; Joshi and Vrieling 2005). With evidence for and against the EICA hypothesis being mixed it can be assumed that our understanding of how botanical invasions occur needs to explore and assimilate other hypothesis.

It is appreciated that community stability is a strong determinant in invasive success, with disturbance and nutrient influx (often human-related) aiding the spread of non-native species, though this theory alone does not explain why non-native plant species should be better at utilising these freed resources (Burke and Grime 1996). Previous experimentation has suggested the importance of enemy release and greater biomass attained in new terrain (Blossey and Nötzold 1995; Leger and Rice 2003), but why this is not the case in all species needs attention. Reconciliation between release from enemy regulation hypothesis (Keane and Crawley 2002) and resource availability hypothesis (Davis, Grime and Thompson 2000) has been proposed, stating that species better able to utilise resources are those who will benefit greatest from enemy release (Blumenthal 2006). Known as the Resource-Enemy Release Hypothesis (R-ERH), this coincides with the observation that successful invaders are often fast growers able to take advantage of greater resource availability quickly, often through plastic traits (Grime 1977; Keane and Crawley 2002). The resolution of these two primary hypotheses also has room for the EICA, though it will act over a longer time frame as evolutionary change in response to enemy release must take place (Blumenthal 2006). In addition, the

importance of generalist and specialist predator release has been touted for further investigation, with decrease in specialist herbivore deterrents observed to correspond with an increased concentration of generalist deterrents, perhaps indicating a trade-off in investment in different deterrents occurring (Joshi and Vrieling 2005).

Conclusions

No support can be found for the EICA hypothesis in *P. lanceolata*, though the results may be influenced by confounding factors such as maternal effects, incomplete enemy release and founder effects (Amsellem *et al.* 2000; Fenner and Thompson 2004). There is doubt that *P. lanceolata* has been present within the USA long enough to exhibit the expected trade-offs (Keane and Crawley 2002). As such, it is not yet possible to state with any certainty whether post-invasion evolution does or does not occur.

These results, along with other studies, indicate that something is missing from the model (Colautti *et al.* 2004). There are numerous models that try to explain why botanical invasions occur, and the EICA hypothesis can be assimilated into these ideas (Blumenthal 2006). However, further research is required to predict what qualities make a plant invasive, and what environmental conditions promote invasion.

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